PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Philippe SANSONETTI et al.	)	Group Art Unit: 1645
	)	
Application No.: 08/466,698	)	Examiner: Albert M. NAVARRO
	)	
Filed: June 6, 1995	)	
	)	
For: METHOD FOR PRODUCING TRANSFORMED *SHIGELLA* (As Amended)	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION OF STEWART THOMAS COLE, PH.D., UNDER 37 C.F.R. § 1.132

I, Stewart Thomas Cole, Ph.D., do hereby make the following declaration:

1. My curriculum vitae, including a list of publications that I have authored or coauthored, is attached hereto as Exhibit A.

2. I have been employed at Institut Pasteur, in Paris, France, since 1983. Since 1989, I have been Chief of the Bacterial Molecular Genetics unit. During the period of 1983-1988, my work was focused on bacterial molecular genetics.

3. On information and belief, attached hereto as Exhibit B is a copy of U.S. patent application Serial No. 08/466,698 ("the '698 application").

4. I have read and understood the contents of the attached copy of the '698 application.

5. On information and belief, the '698 application was filed on June 6, 1995.

6. On information and belief, the '698 application claims the benefit of priority of European Patent Application Serial No. 88 401 842.5, which was filed on July 15, 1988.

7. On information and belief, attached hereto as Exhibit C is a copy of "Proposed Claims for U.S. Patent Application Serial No. 08/466,698" ("the proposed claims").

8. On information and belief, attached hereto as Exhibit D is a copy of a journal article published in the August 15, 1986, issue of Cell, authored by Makino et al., and entitled "A Genetic Determinant Required for Continuous Reinfection of Adjacent Cells on Large Plasmid in *S. flexneri* 2a" ("Makino").

9. I have read and understood the contents of the attached copy of Makino.

10. I am submitting this Declaration to explain the significance of certain terms that appear in the proposed claims to describe modified *Shigella*. In particular, I am submitting this Declaration to explain how these terms in the proposed claims would have been understood on July 15, 1988. In considering the meaning of the terms, I have considered the disclosure of the '698 application.

11. As described in the '698 application, Shigellosis (also known as bacillary dysentery) is a disease endemic throughout the world. (Exhibit B at page

1, lines 14-22.) The primary step in the pathogenesis of bacillary dysentery is invasion of the human colonic mucosa by *Shigella* bacteria. (Exhibit B at page 1, lines 23-24.) Mucosal invasion encompasses many steps, including penetration of the bacteria into epithelial cells, intracellular multiplication, killing of host cells, and finally spreading to adjacent cells and to connective tissue. (Exhibit B at page 1, lines 25-29.) The result is a strong inflammatory reaction that leads to abscesses and ulcerations of the mucosal surface. (Exhibit B at page 1, lines 29-32.) Shigellosis may lead to toxic megacolon, leukemoid reactions and hemolytic-uremic syndrome, which can be fatal. (Exhibit B at page 2, lines 5-8.)

12. The '698 application describes modified strains of *Shigella* that can be used to make a vaccine against a wild strain of *Shigella*. (Exhibit B at page 1, lines 4-13 and page 5, lines 8-34.) Although the '698 application does not use the term, this type of strain was known as of July 15, 1988, as it is today, as a "live attenuated strain". For a live attenuated strain to function effectively as a vaccine, the strain must be modified by mutation of one or more genes to eliminate its pathogenicity, but not the ability of the strain to elicit a protective immune response. Such strains were known as of July 15, 1988. For example, the "International Dictionary of Medicine and Biology", published in 1986, defined an "attenuated vaccine" at page 3083 as "A live bacterial or viral vaccine, carrying mutations that eliminate its pathogenicity but not its ability to elicit a protective immune response." (A copy of the relevant pages of International Dictionary of Medicine and Biology (1986) is attached hereto as Exhibit E.)

13. As of July 15, 1988, it was known that making a live attenuated *Shigella* strain would require modifying a wild *Shigella* strain by mutating one or more genes required for pathogenicity of the wild strain, to create a modified strain that will invade and multiply in a host, but, unlike the corresponding wild strain, will not cause a disease pathology. It was appreciated that, while attenuation of the live attenuated strain is critical to render the strain non pathogenic, it is imperative that the strain retain some ability to invade, multiply, and spread within an inoculated host, so that the strain elicits a significant enough immune response to confer immunity to the wild strain to the host.

14. Proposed claim 24 recites "[a] method for modifying a wild strain of an enteroinvasive *Shigella* to produce a modified strain of *Shigella* that can not spread substantially within infected cells of a host and can not spread substantially from infected to uninfected cells of the host, for use in making a vaccine against the wild strain of *Shigella*. . . ." I interpret this language to mean that the ability of the strain to spread within infected host cells, and from infected to uninfected host cells, is substantially reduced. However, the ability of the strain to spread within infected host cells, and from infected to uninfected host cells, is clearly not abolished. If it were, the modified strain would not be useful to make a vaccine against the wild strain of *Shigella*.

15. Similarly to proposed claim 24, each of proposed claims 36, 37, 54, 55, 74, and 79 recites a modified *Shigella* that "can not spread substantially within infected cells of a host and can not spread substantially from infected to uninfected cells of the host." Each of these claims recites "[a] modified *Shigella* for use in

making a vaccine against a wild strain of *Shigella*." I interpret this language in each of these claims to mean that the ability of the modified strain to spread within infected host cells, and from infected to uninfected host cells, is substantially reduced. However, the ability of the strain to spread within infected host cells, and from infected to uninfected host cells, is clearly not abolished. If it were, the modified strain would not be useful to make a vaccine against the wild strain of *Shigella*.

16. Proposed claim 25, as it depends from proposed claim 24, recites that the modified strain of *Shigella* produced by the method "can not spread substantially within infected cells of a host and can not spread substantially from infected to uninfected cells of the host" and "also can not substantially invade cells of the host." I interpret this language to mean that the ability of the modified strain to spread within infected host cells, the ability of the strain to spread from infected to uninfected host cells, and the ability of the strain to invade host cells are all substantially reduced. However, the ability of the modified strain to spread within infected host cells, the ability of the strain to spread from infected to uninfected host cells, and the ability of the strain to invade host cells, are clearly not abolished. If they were, the modified strain would not be useful to make a vaccine against the wild strain of *Shigella*.

17. Similarly to proposed claim 25, each of proposed claims 36, 37, 54, and 55, recites a modified *Shigella* that "can not spread substantially within infected cells of a host and can not spread substantially from infected to uninfected cells of the host" and "also can not substantially invade cells of the host." Each of

these claims recites "[a] modified *Shigella* for use in making a vaccine against a wild strain of *Shigella*." I interpret this language to mean that the ability of the modified strain to spread within infected host cells, the ability of the strain to spread from infected to uninfected host cells, and the ability of the strain to invade host cells are all substantially reduced. However, the ability of the modified strain to spread within infected host cells, the ability of the strain to spread from infected to uninfected host cells, and the ability of the strain to invade host cells, are clearly not abolished. If they were, the modified strain would not be useful to make a vaccine against the wild strain of *Shigella*.

18. Proposed claim 26, as it depends from proposed claim 25, recites that the modified strain of *Shigella* produced by the method "can not spread substantially within infected cells of a host and can not spread substantially from infected to uninfected cells of the host" and "also can not substantially invade cells of the host" and "also can not produce toxins that kill a substantial number of the host's cells." I interpret this language to mean that the ability of the modified strain to spread within infected host cells, the ability of the strain to spread from infected to uninfected host cells, the ability of the strain to invade host cells, and the ability of the strain to produce toxins that kill a substantial number of the host's cells are all substantially reduced. However, the ability of the modified strain to spread within infected host cells, the ability of the strain to spread from infected to uninfected host cells, the ability of the strain to invade host cells, and the ability of the strain to produce toxins that kill a substantial number of the host's cells, are

clearly not abolished. If they were, the modified strain would not be useful to make a vaccine against the wild strain of *Shigella*.

19. Similarly to proposed claim 26, each of proposed claims 37 and 55 recites a modified *Shigella* that "can not spread substantially within infected cells of a host and can not spread substantially from infected to uninfected cells of the host" and "also can not substantially invade cells of the host" and "also can not produce toxins that kill a substantial number of the host's cells." Each of these claims recites "[a] modified *Shigella* for use in making a vaccine against a wild strain of *Shigella*." I interpret this language to mean that the ability of the modified strain to spread within infected host cells, the ability of the strain to spread from infected to uninfected host cells, the ability of the strain to invade host cells, and the ability of the strain to produce toxins that kill a substantial number of the host's cells are all substantially reduced. However, the ability of the modified strain to spread within infected host cells, the ability of the strain to spread from infected to uninfected host cells, the ability of the strain to invade host cells, and the ability of the strain to produce toxins that kill a substantial number of the host's cells, are clearly not abolished. If they were, the modified strain would not be useful to make a vaccine against the wild strain of *Shigella*.

20. I have read the Declaration of Jean-Michel Alonso, M.D., Ph.D., Under 37 C.F.R. § 1.132, a copy of which is attached hereto as Exhibit F. I agree with Dr. Alonso that, based on the teachings of Makino, I would not expect that a modified *Shigella* strain comprising an inactivated *icsA* gene would be useful as a live attenuated strain for making a vaccine against the wild *Shigella* strain.

21. In reaching this conclusion, I am aware that Makino states that modified *Shigella* strains comprising an inactivated *icsA* gene "may be a plausible candidate for a live vaccine against bacillary dysentery." (Exhibit D at page 554, left col.) I disagree with this statement. In fact, this assertion is clearly contrary to the description of the modified *Shigella* strain comprising an inactivated *icsA* gene provided by Makino. According to Makino, the modified strain is unable to survive in cells or tissues and does not spread within or between cells. (Exhibit D at page 551, right col. and page 554, left col.) For this reason, the strain would not elicit a robust immune response and would not be effective for making a vaccine.

22. Based on the disclosure in Makino, and based on what was known as of July 15, 1988, and absent reading the disclosure of the '698 application, I would not have been motivated to include an inactivated *icsA* gene in a modified *Shigella* strain for use in making a vaccine. To the contrary, I would have assumed that inclusion of an inactivated *icsA* gene in such a strain would have rendered it ineffective in making a vaccine against a wild strain of *Shigella*.

23. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the

application or any patent issuing thereon.

Dated: 16.1.2004

By: _____
Stewart Thomas Cole, Ph.D.